Mass Spectrometry 101

Hackert - CH 370 / 387D

Based in part on Lecture Notes from
"An Introductory Lecture On Mass Spectrometry Fundamentals"
Presented to the Sandler Mass Spectrometry Users' Group,
University of California San Francisco, and
"Fundamentals of Mass Spectrometry – Based Proteomics"
by Doug Sheeley – Division of Biomedical Technology, National Center for Research Resources

What does a mass spectrometer do?

1. It measures mass **(m/z)** better than any other technique.

2. It can give information about chemical structures.

What are mass measurements good for?

To identify:

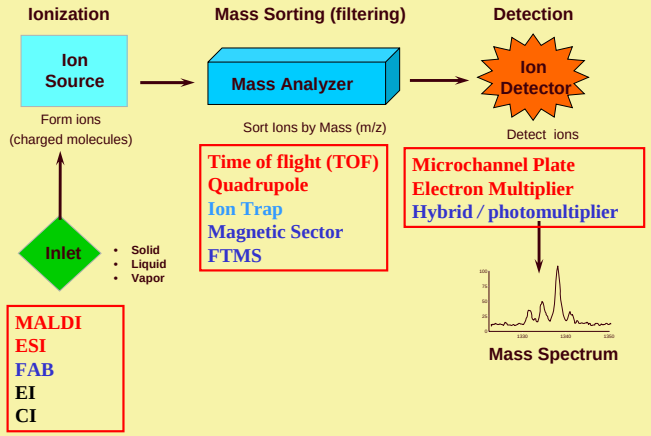
- metabolites, synthetic organic chemicals
- peptides, proteins, recombinant proteins,
- oligonucleotides, polymers
- drug candidates,
- complexes

,

Who uses mass measurements?

- Pharmaceutical analysis
  - Bioavailability studies
  - Drug metabolism studies, pharmacokinetics
  - Characterization of potential drugs
  - Drug degradation product analysis
  - Screening of drug candidates
  - Identifying drug targets
- Biomolecule characterization
  - Proteins and peptides
  - Oligonucleotides
- Environmental analysis
  - Pesticides on foods
  - Soil and groundwater contamination
- Forensic analysis/clinical

Summary: acquiring a mass spectrum



Mass Spectrometry – Focus on Proteomics

Source: produces charged particles (ions)

- **Electron Impact (EI) - Hard (fragments) / 1000 Da**
- Chemical Ionization (CI) – (methane / isobutane / ammonia)
- Fast Atom Bombardment (FAB) – 6keV xenon atoms
- **Electrospray Ionization (ESI) - Soft / 200kDa**
- **Matrix-Assisted Laser Desorption Ionization - Soft / 500kDa**

Mass Spectrometry

Introductory Example: mass spectrum of water

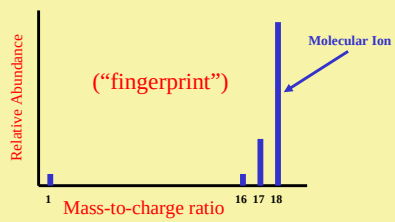
- **Electron Impact (EI) - Hard (fragments) / 1000 Da**

H_2O + fast electron → $[H_2O]+$ + 2 electrons

+ fragments ([OH]+, O+, H+)

Fragmentation pattern

$[H_2O]+$	**18**
[OH]+	**17**
O+	**16**
H+	**1**



How is mass defined?

Assigning numerical value to the intrinsic property of "mass" is based on using **carbon-12, ^{12}C**, as a reference point.

One unit of mass is defined as a **Dalton (Da).**

One Dalton is defined as 1/12 the mass of a single carbon-12 atom.

Thus, **one ^{12}C atom has a mass of 12.0000 Da**.

Isotopes

+Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, **1.1% of C atoms have an extra neutron, making their mass 13 Da.**

+Why do we care?

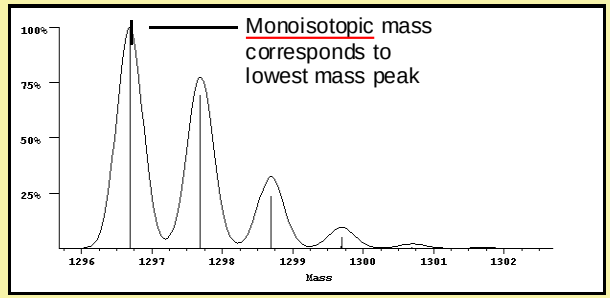
Mass spectrometers can "see" isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

Stable isotopes of most abundant elements of peptides

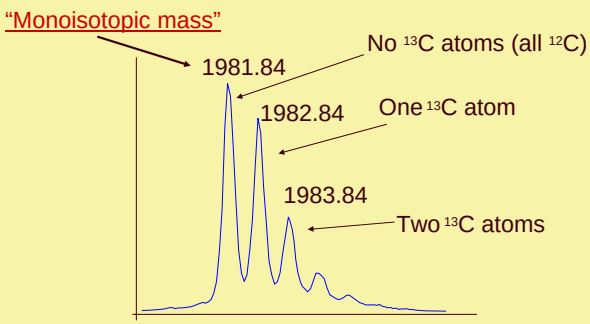
Element	Mass	Abundance
H	1.0078 2.0141	99.985% 0.015
C	12.0000 13.0034	98.89 1.11 ←
N	14.0031 15.0001	99.64 0.36 ←
O	15.9949 16.9991 17.9992	99.76 0.04 0.20 ←

Monoisotopic mass

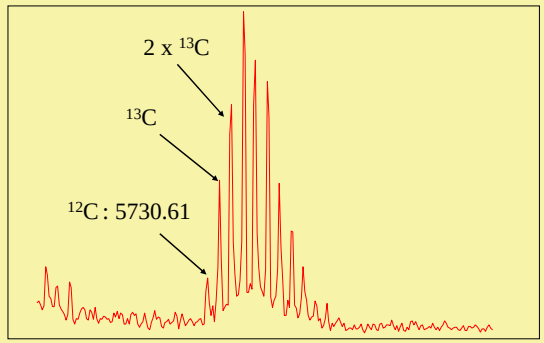


When the isotopes are clearly resolved the **monoisotopic mass** is used as it is the most accurate measurement.

Mass spectrum of peptide with 94 C-atoms (19 amino acid residues)

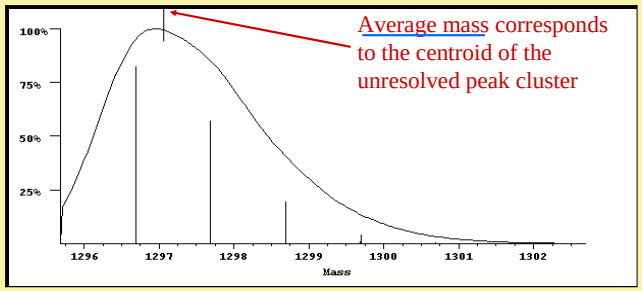


Mass spectrum of insulin



Insulin has 257 C-atoms. Above this mass, the monoisotopic peak is too small to be very useful, and the average mass is usually used.

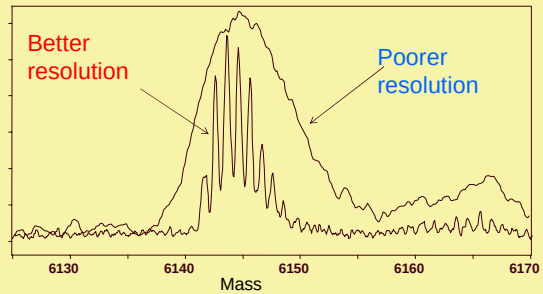
Average mass

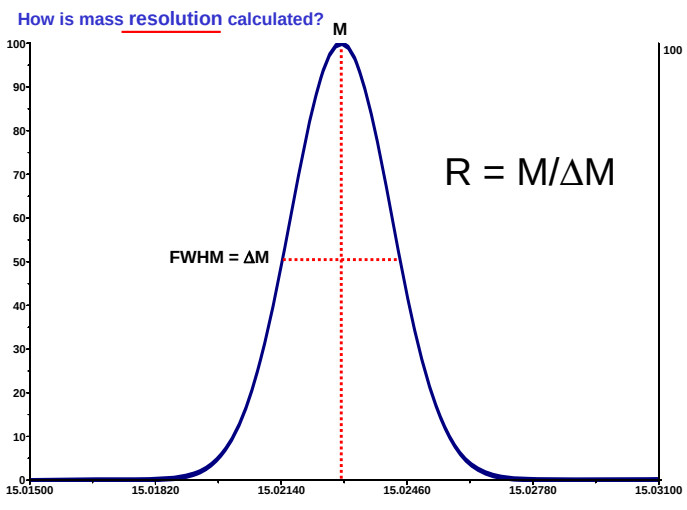


When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the the isotope peaks in the cluster, which is the same as the average or chemical mass.

What if the resolution is not so good?

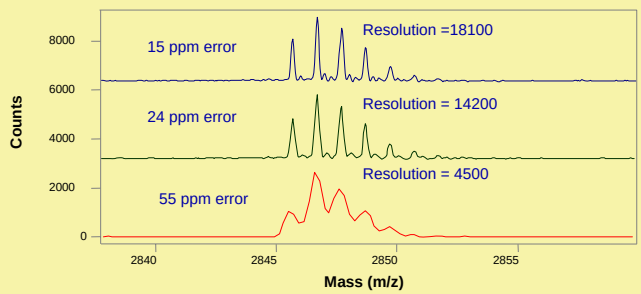
At lower resolution, the mass measured is the average mass.



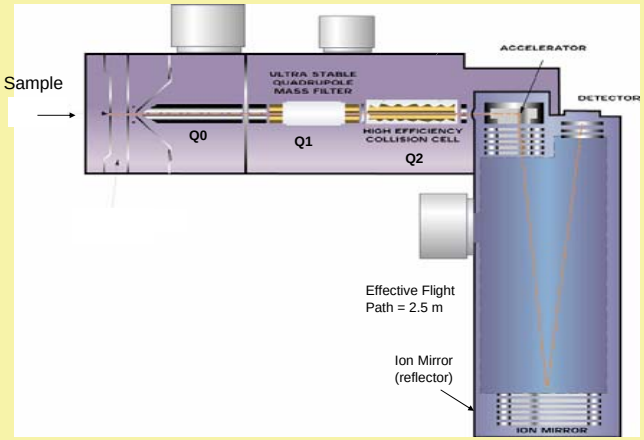


Mass measurement accuracy depends on resolution

High resolution means better mass accuracy



ESI QQ TOF or MALDI QQ TOF



How do mass spectrometers get their names?

Types of ion sources:

- **Electrospray** (ESI) - **Soft / 200kDa**
- **Matrix Assisted Laser Desorption Ionization** (MALDI) **~ 500kDa**

Types of mass analyzers:

- **Quadrupole (Quad, Q)**
- **Time-of-Flight (TOF)**
- Ion Trap

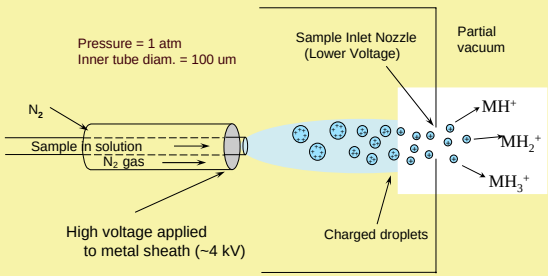
-Either source type can work with either analyzer type: "MALDI-TOF," "ESI-Quad."

-Analyzers can be combined to create "hybrid" instruments. ESI-QQQ, MALDI QQ TOF, Q Trap

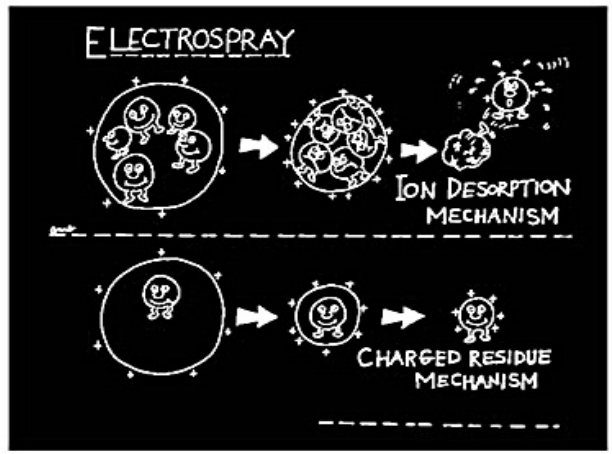
Ion Sources make ions from sample molecules

(Ions are easier to detect than neutral molecules.)

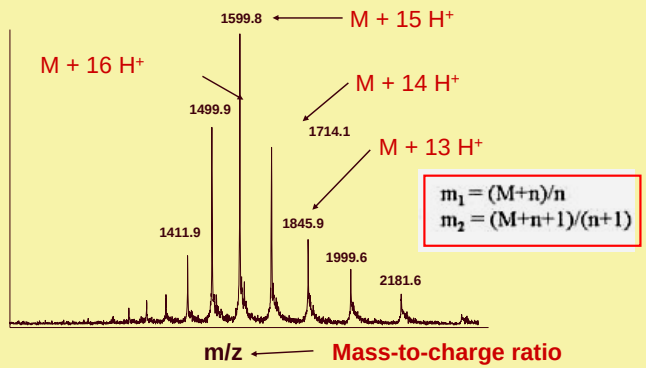
Electrospray ionization:



Very gentle / solvent evaporates / many, multiple charges (H+)

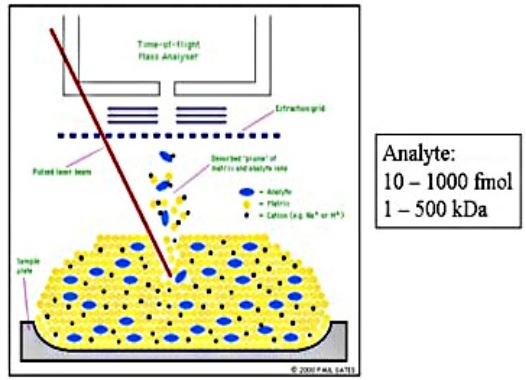


ESI Spectrum of Trypsinogen (MW 23,983)

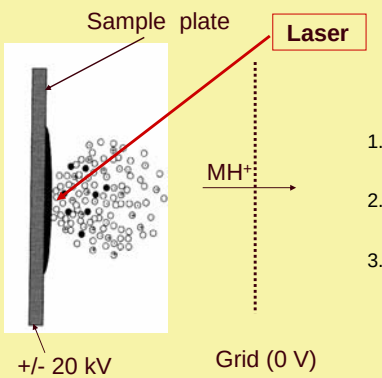


MALDI (Matrix Assisted Laser Desorption Ionization)

"Nuke It"

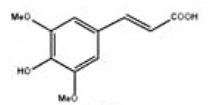


MALDI: Matrix Assisted Laser Desorption Ionization

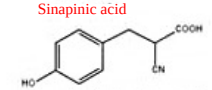


1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer: $XH^+ + M \rightarrow MH^+ + X$.

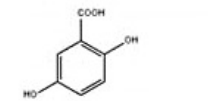
MALDI-TOFMS



Sinapinic acid



Cyano-4-hydroxy-trans –cinnamic acid



2,5-Dihydroxybenzoic acid

the three most commonly used matrices

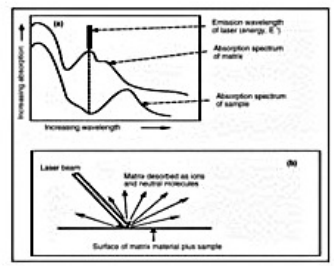
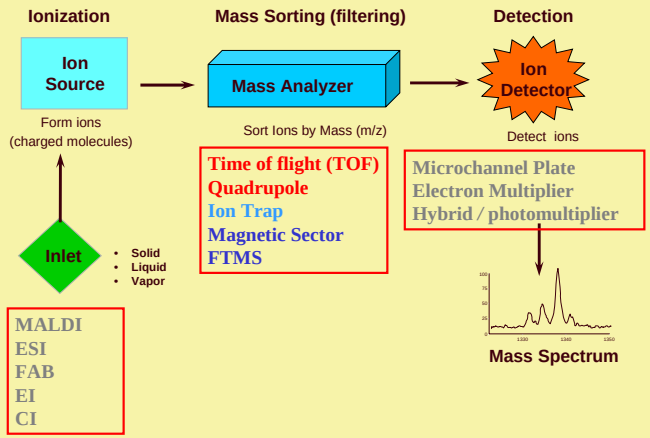


Figure 3 In a MALDI experiment, the sample is mixed or dissolved in a matrix material, which has an absorption spectrum that matches the laser wave length of energy, E'. The sample may not have a matching absorption peak (a) but this is not important because the matrix material absorbs the radiation, some of which is passed on to the dissolved sample.
Neutral molecules and ions from both sample and matrix material are desorbed (b).

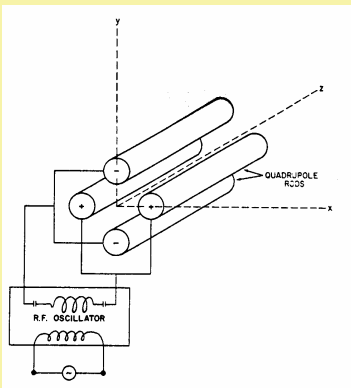
Summary: acquiring a mass spectrum



Mass analyzers separate ions based on their mass-to-charge ratio (m/z)

- Operate under **high vacuum** (keeps ions from bumping into gas molecules)
- Actually measure **mass-to-charge ratio** of ions **(m/z)**
- Key specifications are resolution, mass measurement accuracy, and sensitivity.
- Several kinds exist: for bioanalysis, quadrupole, time-of-flight and ion traps are most used.

Quadrupole Mass Analyzer



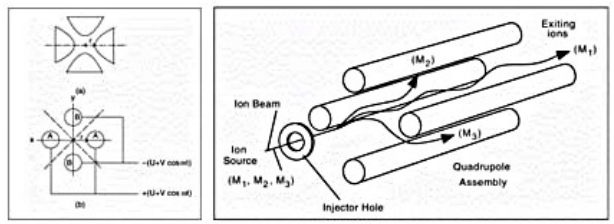
Uses a combination of RF and DC voltages to operate as a mass filter.

- Has four parallel metal rods.
- Lets one mass pass through at a time.
- Can scan through all masses or sit at one fixed mass.

Mass Analyzers: The Quadrupole Mass Filter

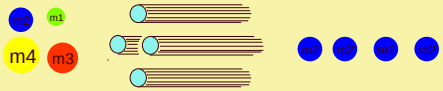
A potential of ~100-1000 V is applied alternately to the opposing pairs of rods at a frequency of a few MHz. At a specific combination of DC & RF, an m/z has a stable trajectory through the rods, and all other m/z are lost. The mass range is scanned as the voltages are swept from min to max, but at constant DC/RF ratio.

Faster Scanning than sector instruments (but not as fast as ion traps or TOF).
Mass Range generally m/z 0-2000 or 0-4000.
Facile MS/MS using Triple Quadrupole (Q-q-Q) analyzer.
Exquisitely sensitive in selected ion monitoring (both analyzers parked at one m/z).
Largely replaced by the ion trap and hybrid Q-q-TOF for biopolymer analysis.



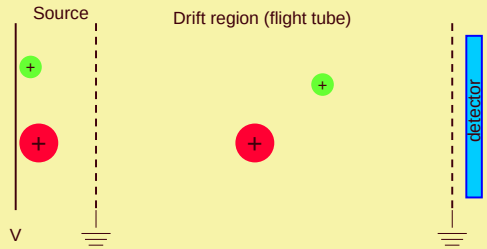
Quadrupoles have variable ion transmission modes

mass scanning mode

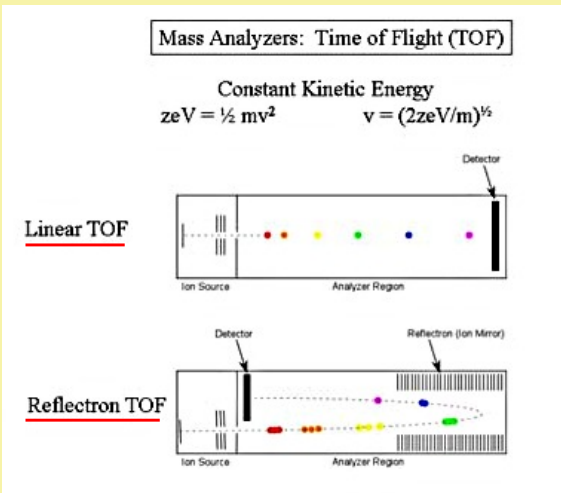


single mass transmission mode

Time-of-flight (TOF) Mass Analyzer

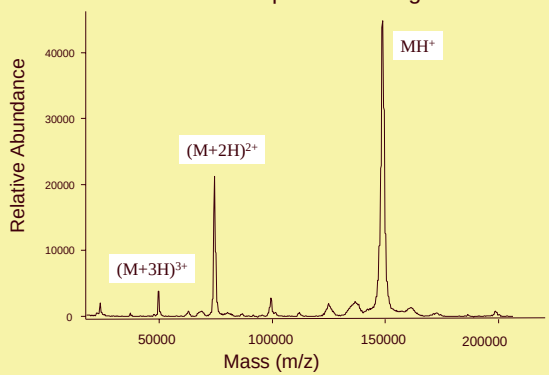


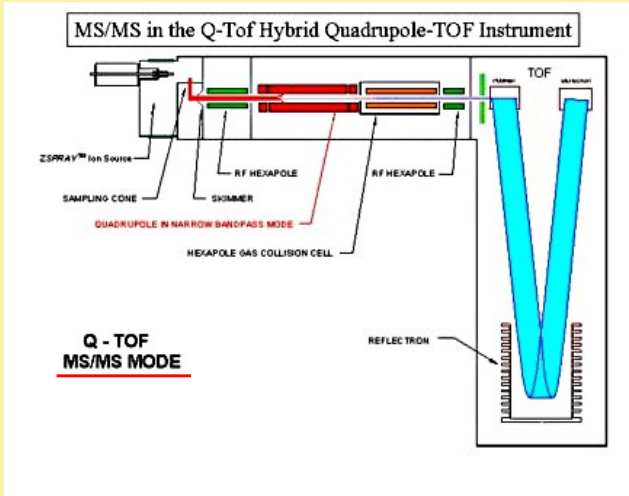
- Ions are formed in pulses.
- The drift region is field free.
- Measures the time for ions to reach the detector.
- Small ions reach the detector before large ones.



The mass spectrum shows the results

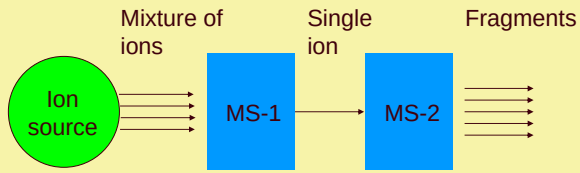
MALDI TOF spectrum of IgG

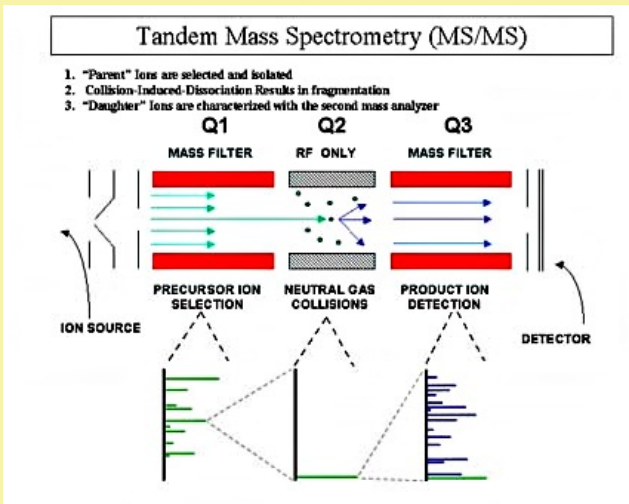




What is MSMS?

MS/MS means using two mass analyzers (combined in one instrument) to select an analyte (ion) from a mixture, then generate fragments from it to give structural information.

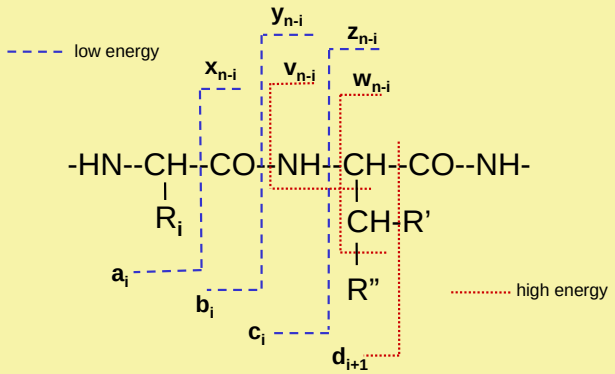




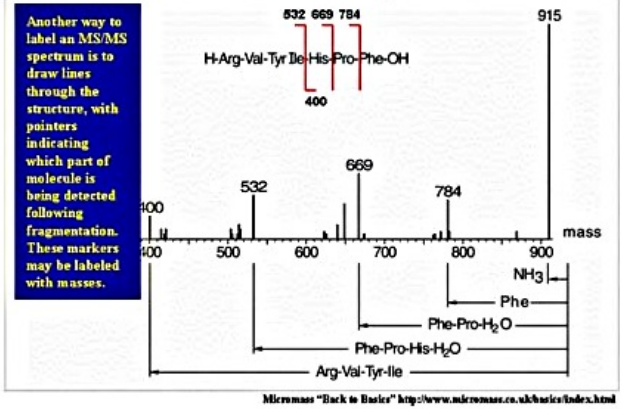
Tandem Mass Spectrometry (MS/MS) is the Method of Choice for Sequence Analysis of Peptides

Speed
Sensitivity
Tolerance for Amino-terminal Blocking Groups
High Specificity for Protein Identification

Cleavages Observed in MS/MS of Peptides

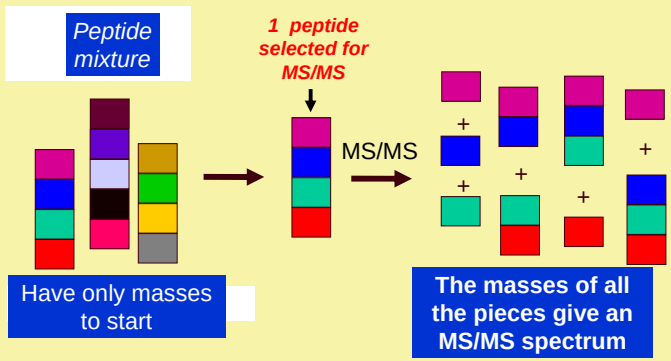


MS/MS of Angiotensin III:
selection and fragmentation of the $(M+H)^+$ molecular ion at m/z932

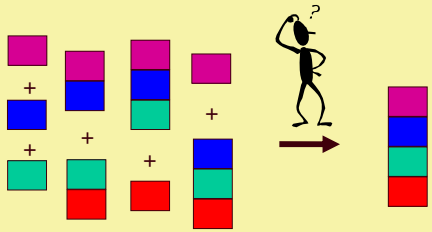


Tryptic Digest of ADH: Expected Peptides vs. Those Detected

What is MS/MS?

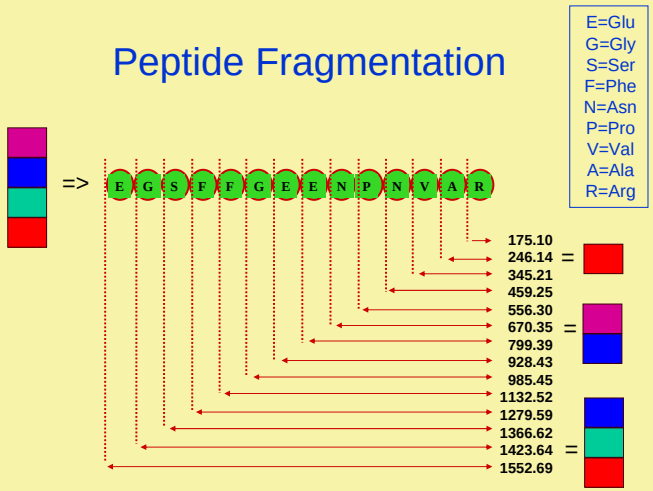


Interpretation of an MSMS spectrum to derive structural information is analogous to solving a puzzle



Use the fragment ion masses as specific pieces of the puzzle to help piece the intact molecule back together

Peptide Fragmentation



Fundamentals of Mass Spectrometry – Based Proteomics

Doug Sheeley
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National Center for Research Resources

Fundamentals of Mass Spectrometry – Based Proteomics

Purpose:
To convey basic concepts in proteomics and biological mass spectrometry, in order to build a working vocabulary and a basis for further study

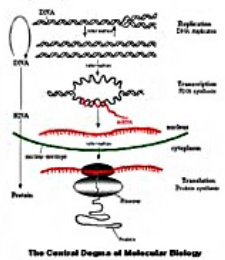
Outline:
1. Proteomics
2. Mass Spectrometry- ion sources and mass analyzers
3. Protein Chemistry in the context of proteomics
4. MALDI-TOF/MS for Peptide Mass Mapping
5. LC/MS/MS for Peptide Sequence Analysis

The Proteome

All an organism's cells carry the same Genome, and it is Static. Genomes do not describe function. They are a parts list.

Different cells express different proteins. The type and quantity of this expression changes.

The Proteome is Dynamic. It is the total of all proteins expressed by a particular *cell* at a given *time*, under specific *conditions*.



A Proteome cannot be studied the way a Genome is sequenced. There has to be a specific biological question behind an experiment. The questions may be either very broad or strictly defined.

Two Dimensional Gel Electrophoresis

Isoelectric focusing is performed on precast gel strips using commercial instruments. Many pH ranges are available. Multiple strips can be run in parallel.

An immobilized pH gradient is created in a polyacrylamide gel strip by incorporating a gradient of acidic and basic buffering groups when the gel is cast.

Resolution is determined by the slope of the pH gradient and the field strength.

Loading capacity depends on gel size and thickness.

In 2D IEF/PAGE, the gel strip from IEF is loaded into a single large well.

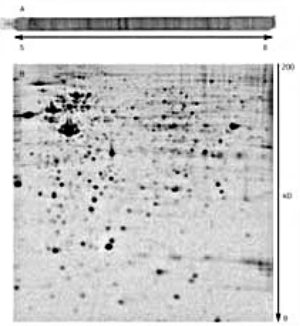


Fig. 1. Principle of 2-D electrophoresis. A, pre-B lymphoma cell extract (1 mg) was separated by IEF on a ReadyStrip pH 5–8 IPG strip, and stained with Bio-Safe™ Coomassie stain. B, Equilibrated strip was run in the second dimension by SDS-PAGE (12% acrylamide). The gel was stained with Coomassie Blue.

Figure from BioRad Product Literature